Biochem. J. (1982) **208**, 147–151
Printed in Great Britain

The differing responses of four muscle types to dexamethasone treatment in the rat

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(*Received 9 July 1982/Accepted 27 July 1982*)

The glucocorticoid dexamethasone dramatically altered growth patterns in four muscle types, inducing atrophy of smooth and fast-twitch skeletal muscle, suppressing protein accumulation in slow-twitch muscle and enhancing growth in the heart. These differing responses were explained by steroid-induced changes in RNA content, protein synthesis and protein breakdown.

The increased secretion of steroids from the adrenal cortex represents part of the acute physiological response to stress. However, large and more prolonged increases in the circulating concentrations of glucocorticoid hormones can be found with infections, physical (Dallman & Jones, 1973) and psychological (Pollard *et al.*, 1976) stresses, in Cushing's syndrome and in various clinical treatments (Leung & Munck, 1975). Although the overall effect on the body is usually catabolic (Long *et al.*, 1940; Loeb, 1976; Tomas *et al.*, 1979), the various body tissues respond quite differently to the glucocorticoids (Baxter & Forsham, 1972; Leung & Munck, 1975). The liver often increases in mass, whereas the musculature as a whole undergoes atrophy. The response of individual muscle types within the musculature is, however, much less clearly defined. We report here very different patterns of growth and associated changes in protein turnover for the smooth muscle of the small intestine and three striated muscles of the rat after 5 days treatment with the synthetic glucocorticoid dexamethasone. As an anti-inflammatory agent this synthetic steroid is approx. 40 times more potent than cortisone. Hence the steroid treatment employed here is roughly equivalent in potency to doses of cortisone or corticosterone (i.e. 100 mg/kg body wt. per day) often used in other animal studies (Goldberg, 1969; Shoji & Pennington, 1977; Rannels *et al.*, 1978; Tomas *et al.*, 1979; Santidrian *et al.*, 1981).

Experimental

Male rats (CD strain; Charles River U.K. Ltd., Manston, Kent, U.K.) initially weighing 200 g were divided into three groups of six. The first group were killed immediately and their muscles analysed as part of the growth-rate determinations (i.e. day 0). The two remaining groups of animals were given five daily subcutaneous injections of either physiological saline (0.9% NaCl; controls) or dexamethasone (2.5 mg/kg per day). Immediately before they were killed on day 5, unanaesthetized animals were injected intravenously with 150 μmol of phenylalanine, containing 65 μCi of L-[4-^{3}H]phenylalanine (sp. radioactivity 24 Ci/mmol; The Radiochemical Centre, Amersham, Bucks., U.K.) in 1 ml of 0.9% NaCl/100 g body wt. At 10 min after the injection commenced, animals were decapitated, bled for 15 s and the appropriate muscles were very rapidly dissected (under ice-cold saline where necessary to prevent further metabolism) and frozen in liquid nitrogen. The entire small intestine (i.e. beginning of duodenum to end of ileum) was simultaneously removed, flushed with ice-cold saline and its length measured. The preparation was slit along its length and the mucosa carefully removed, as previously described (McNurlan *et al.*, 1979).

Protein synthesis was measured in each muscle by the method of McNurlan *et al.* (1979), with the specific radioactivity of free phenylalanine in the intracellular pool (S_A) or covalently bound in protein (S_B) being measured after prior hydrolysis of the tissue and conversion of phenylalanine into β-phenethylamine (Garlick *et al.*, 1980). The fractional rate of synthesis (K_s) was determined by

$$K_s = \frac{S_B}{S_A t} \times 100$$

where t is the time expressed in days. In the original method (McNurlan *et al.*, 1979), S_A was given as a mean value (i.e. at 5 min) derived from measurements at both 2 and 10 min. In our study S_A was used at 10 min only, since in these muscles we found

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this value to differ by less than 5% from that measured at 2 min.

Daily growth rates (K_g) in each tissue were determined as a percentage of the protein mass that had been accumulated, or lost, over the experimental period (i.e. between days 0 and 5), divided by the mean protein content (at 2.5 days). The total amount of protein synthesized in the muscle was calculated as the product of the fractional synthetic rate and the protein content at day 5. Since the protein mass is regulated by the relative rates of synthesis and breakdown, the fractional rate of protein breakdown (K_b) was calculated by subtracting the measured growth rate (K_g) from the measured fractional rate of synthesis (K_s), i.e. $K_b = K_s - K_g$.

Results and discussion

The dexamethasone-treated rats lost weight in a linear manner over the 5 days studied. Hence, instead of growing at approx. 2% per day, these animals lost weight at an equivalent rate. This weight loss could not be explained by any significant change in the animals' daily food intake (200 mg/g body wt.). Any subsequent malabsorption across the gastrointestinal tract in response to the steroid treatment cannot, however, be ruled out.

Consistent with the known detrimental effects of the glucocorticoids on the gut was a marked atrophy of the smooth muscle of the small intestine. This was evident as both a large loss in total protein (Table 1) and a 10% decrease in the non-stretched length of the small intestine. Although dexamethasone significantly decreased the total amount of protein being synthesized in the smooth muscle (i.e. $K_s \times$ protein content), this was not due to any significant change in the fractional rate of protein synthesis (K_s), but rather reflected the diminished protein mass of this tissue (Table 1). In consequence, the profound wasting of the smooth muscle after exposure to the steroid must have occurred through an elevated turnover of its proteins, as was indeed indicated by the increased fractional rate of breakdown, K_b (Table 1). Hence, in this smooth-muscle preparation the main action of dexamethasone appeared to be on protein breakdown and not on synthesis. A previous report (McNurlan & Garlick, 1981) on diabetic animals also indicated a possible preferential effect on breakdown (presumably decreased) in connection with the enhanced growth of the jejunal serosa in the absence of any change in the synthetic rate.

In direct contrast with the smooth muscle, the growth rate of the heart was increased 2-fold after 5 days of dexamethasone treatment, compared with

Table 1. *Dexamethasone-induced changes in the protein mass and protein turnover of four different muscle types*
Each value is the mean ± S.E.M. for at least six muscles, with the percentage differences between these values shown in parentheses. Where possible, statistical differences between the means of 5-day controls and dexamethasone-treated (2.5 mg/kg per day) muscles were determined by using Student's t test (*$P < 0.01$; **$P < 0.025$; NS, not significant). The smooth-muscle preparation of the small intestine consists of the muscularis externa and serosa. K_g and K_g†† are the growth rates derived from the changes in the protein mass between days 0 and 5 and days 0 and 1, respectively. K_g was used in the subsequent calculations of the rate of breakdown (K_b).

	Protein content (mg) at		Daily growth rate		Fractional rate of synthesis	Total protein synthesized	Fractional rate of breakdown
	Day 0	Day 5	(K_g)	(K_g††)	(K_s)	(mg/day)	(K_b)
Smooth muscle							
Control	1256 ± 27	1380 ± 32	1.9	1.9	82 ± 1.7	1132 ± 49	80.1
+ Dexamethasone		799 ± 28	−9.5	−15.4	83 ± 5.3	663 ± 37	92.5
		(−42*)			(+1, NS)	(−42*)	(+15)
Tibialis anterior							
Control	66 ± 3.5	72 ± 1.9	1.7	1.4	9.1 ± 0.4	6.5 ± 0.3	7.4
+ Dexamethasone		61 ± 1.3	−1.6	−2.7	5.7 ± 0.5	3.5 ± 0.3	7.2
		(−15*)			(−37*)	(−46*)	(−2)
Soleus							
Control	16 ± 0.4	18 ± 0.8	2.4	2.5	14.8 ± 0.7	2.9 ± 0.1	12.4
+ Dexamethasone		17 ± 0.3	1.0	0.8	13.8 ± 0.7	2.4 ± 0.2	12.8
		(−6**)			(−7, NS)	(−17**)	(+4)
Heart							
Control	129 ± 1.4	140 ± 3.1	1.6	2.2	13.3 ± 0.5	18.6 ± 0.3	11.7
+ Dexamethasone		152 ± 2.4	3.3	3.9	11.4 ± 0.5	17.3 ± 0.3	8.1
		(+9**)			(−14, NS)	(−7, NS)	(−30)

controls (Table 1). Interestingly, in cardiac muscle also, neither the fractional nor the total rates of synthesis were significantly changed by the steroid. Similar steroid treatments have also been found to be ineffective in changing the protein-synthetic rates of perfused hearts (Rannels *et al.*, 1978). Hence in both smooth and cardiac muscle the action of dexamethasone seemed to be primarily directed against protein degradation. However, in contrast with the increased fractional rate of protein breakdown in the wasting smooth muscle, the fractional rate of degradation was inhibited in cardiac muscle; this latter change correlated with the additional growth of the heart after exposure to this steroid (Table 1). This steroid-induced inhibition of protein breakdown was subsequently confirmed when protein degradation *in vitro* was measured on ventricular slices prepared from control and steroid-treated hearts (F. J. Kelly & D. F. Goldspink, unpublished work).

The dexamethasone-induced changes in the two skeletal muscles were again different, both from those in the smooth and cardiac muscle and from each other. In the slow-twitch soleus muscle, growth was merely slowed by exposure to dexamethasone. In contrast, in the same steroid-treated animals a pronounced atrophy of the fast-twitch tibialis anterior was found (Table 2). The magnitude of these changes in skeletal muscle was intermediate with respect to those induced in cardiac and smooth muscle. Previous studies have shown similar differential effects of glucocorticoids on the growth (Goldberg, 1969; Shoji & Pennington, 1977; Kelly & Goldspink, 1981), morphology (Vignos *et al.*, 1976) and certain physiological parameters (Gardiner *et al.*, 1980) of fast- and slow-twitch skeletal muscles. However, such differences in response have not been clearly defined in terms of the steroids' action(s) *in vivo* on both protein synthesis and protein breakdown, and particularly within individual skeletal muscles. The slowing of growth in the soleous correlated with small complementary changes in the fractional rates of both protein synthesis and breakdown (Table 1), neither of which alone was statistically significant. In contrast, in the tibialis anterior the steroid appeared to exert its catabolic effect by markedly inhibiting the synthesis of new proteins, without altering the fractional rate of degradation (Table 1).

Table 2. *Dexamethasone-induced changes in the RNA content and protein synthesis per unit of RNA*

RNA was extracted and measured (Goldberg & Goldspink, 1975) in the same muscles in which protein synthesis was measured (Table 1). Percentage differences between control and dexamethasone-exposed muscles are given in parentheses (*$P < 0.001$; NS, not significant).

	Total RNA P (μg)	Protein synthesized (mg/mg of RNA P per day)
Smooth muscle		
Control	5424 ± 189	209 ± 12
+Dexamethasone	3657 ± 94	181 ± 18
	(−33*)	(−13, NS)
Tibialis anterior		
Control	53 ± 1.3	120 ± 6
+ Dexamethasone	27 ± 1.0	127 ± 9
	(−49*)	(+6, NS)
Soleus		
Control	17 ± 0.8	186 ± 17
+ Dexamethasone	12 ± 0.6	200 ± 14
	(−31*)	(+8, NS)
Heart		
Control	105 ± 3.5	177 ± 9
+ Dexamethasone	99 ± 4.2	175 ± 12
	(−6, NS)	(−2, NS)

A glucocorticoid suppression of protein synthesis in skeletal muscle has previously been found in a variety of studies, both *in vitro* and *in vivo*. However, most investigations *in vivo* have not analysed single muscles, but by virtue of pooling anatomically related muscles have in fact mixed various skeletal-muscle types. Nonetheless our findings *in vivo* (Table 1) are in good agreement with those from perfused hemicorpus preparations (Rannels & Jefferson, 1980) in indicating that the synthetic rate of the fast-twitch muscles are more markedly inhibited by glucocorticoid hormones.

Steroid-induced changes, if any, in protein breakdown in different types of skeletal muscle remain poorly defined. No discernible effects on breakdown were found *in vivo* in either fast- or slow-twitch muscles, and as such confirms data from hemicorpus preparations (Rannels & Jefferson, 1980). It does, however, contrast with another study from our laboratory which describes a direct (*in vitro*) inhibitory action of various glucocorticoids (over 4h) on protein breakdown measured in isolated soleus muscles (McGrath & Goldspink, 1982). Such apparent differences are currently unexplained, but possibly relate to the muscle's mechanical activity and/or the glucocorticoids' permissive actions, which, although present *in vivo*, are absent *in vitro*, and which are probably important determinants of the tissue's responsiveness to these steroids (see below).

Clearly the four muscle types respond very differently upon exposure to dexamethasone and thus contribute to different extents to the general loss in body weight. In both smooth and cardiac muscle changes in growth appeared to occur principally through alterations in protein breakdown rather than synthesis. Except for the regenerating liver (Scornik & Botbol, 1976) and the soleus muscle after

dynamic exercise (Watt *et al.*, 1982), this is, to our knowledge, the only situation where changes in breakdown alone have been reported, and serves to re-emphasize the importance of protein degradation in regulating tissue growth. Although steroid-induced increases in the urinary excretion of *N*-methylhistidine (Tomas *et al.*, 1979; Santidrian *et al.*, 1981) suggest an increased breakdown of myofibrillar proteins from the musculature, this technique tells us nothing of the events occurring within individual muscles or the different muscle types. Of the four types studied here, only smooth muscle showed an elevated breakdown of proteins in response to dexamethasone. To obtain meaningful measurements of protein breakdown in individual muscles *in vivo* is technically difficult. For example, the decay of prelabelled proteins is likely to give erroneous measurements, owing to the efficient reutilization of most tracer amino acids and the non-exponential decay of label in mixed proteins (Waterlow *et al.*, 1978). In turn, the accuracy of our calculated values of breakdown (K_b) depends on the accuracy of the measured rates of synthesis and tissue growth. Of the two, the latter is the most likely to be in error (Waterlow *et al.*, 1978). We therefore restricted the experimental period, and measurement of growth rates, to 5 days to minimize any daily fluctuations in growth. For the most part, similar changes in the daily growth rates were also found over shorter periods (24h) of the steroid treatment (see K_g^{++} in Table 1). These data, together with a linear change in body weight, suggest a uniform response with time. The only possible exception to this may be found in the smooth muscle. Its initial atrophy after exposure to dexamethasone may be more rapid than that measured over the longer period of 5 days (i.e. compare K_g^{++} and K_g in Table 1). If this is the case, the steroid-induced increase in protein breakdown (as calculated from K_g, Table 1) will, if anything, be underestimated. The synthetic rates in the control muscles are also in good agreement with other published values (Waterlow *et al.*, 1978; McNurlan & Garlick, 1981; Moalic *et al.*, 1981; Watt *et al.*, 1982), with the turnover and renewal of proteins being much higher in the smooth muscle, compared with that in the striated tissues (Table 1; Garlick *et al.*, 1980).

The dexamethasone-induced changes in the synthesis of new proteins could arise from alterations in either the total amount of RNA in the tissue or in the activity of RNA in the translation process (i.e. synthesis/unit of RNA). After 5 days of dexamethasone treatment the suppressed total synthesis of proteins in all muscles (except the heart) correlated with commensurate decreases in the RNA content, protein synthesis per unit of RNA remaining unchanged (Table 2). No significant changes were found in either the RNA content or protein synthesis per unit of RNA in steroid-treated cardiac muscle (Table 2). This was not, however, surprising, since the additional growth of the heart was not accomplished by changing protein synthesis (Table 1).

Both the heart and soleus muscle exhibit high degrees of contractility in fulfilling their functions within the body, and their activity patterns may afford these tissues some resistance to the catabolic actions of this steroid. In contrast, fast-twitch muscles, such as the tibialis anterior, are less frequently recruited and were clearly more susceptible to the hormone's actions (Table 1; Goldberg, 1969; Rannels & Jefferson, 1980; Kelly & Goldspink, 1981). Although contactile activity is probably one important determinant of a muscle's responsiveness to steroid hormones (Goldberg, 1969; McGrath & Goldspink, 1978; Goldspink, 1980), it alone cannot explain all of the different effects produced by dexamethasone, particularly since smooth muscle in the small intestine exhibits a continuous form of spontaneous activity.

A direct action of dexamethasone and other glucocorticoids on protein turnover has been clearly shown in isolated muscle preparations (Kostyo & Redmond, 1966; McGrath & Goldspink, 1978, 1982). However, the injected dexamethasone may also cause several metabolic, endocrine (Tomas *et al.*, 1979) and electrolyte changes within the body, which in turn may indirectly act to varying degrees on the four muscle types studied. For example, the hypertensive effects of glucocorticoid hormones (Knowlton *et al.*, 1952) may subject the heart to mechanical overload, with such additional demands being met by the compensatory growth of the heart. Protein synthesis may also be enhanced directly in the heart, but not in skeletal muscle, by transient increase in plasma fatty acids and ketone bodies (Jefferson *et al.*, 1974). Such changes may cancel out any direct inhibitory action that the steroid might have on protein synthesis in cardiac muscle. Hence, the observed changes in protein turnover in the heart could possibly be the net result of a complex interaction of direct and indirect (chemical and mechanical) effects produced in response to the exogenous steroid. Such possible direct and indirect actions of this steroid on the heart require further study.

We thank Paul Anderson, B.Sc., and Mrs. Gwen Sanderson for their excellent technical assistance, the British Heart Foundation for their financial support and Dr. P. J. Garlick, Dr. M. A. McNurlan and Dr. V. K. Preedy (Clinical Nutrition and Metabolic Unit, London School of Hygiene and Tropical Medicine) for generously allowing us access to their method for measuring protein synthesis before its publication. F. J. K. was the recipient of a studentship from the Department of Education in Northern Ireland.

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